WJEC Wales Biology A Level

SP 1.4: Investigation into the effect of temperature on enzyme activity

Practical notes

Introduction

The rate of an enzyme-controlled reaction is influenced by different **factors** including temperature, pH, enzyme concentration and substrate concentration. The effect of each of these factors can be determined by changing **one variable only**, and observing its effect on the reaction rate.

In this practical, we will determine the effect of **temperature** on **amylase activity**.

The enzyme **amylase catalyses** the hydrolysis of **starch**. The reaction can be followed by adding **samples** of the reaction mixture to **iodine** at set time intervals.

Iodine turns from a **yellow-brown** to a **blue-black** colour in the presence of starch. At the start of the reaction the high concentration of starch will turn the iodine blue-black. As the reaction proceeds, starch is used up and the colour gets lighter. When no colour change is observed, the reaction is complete.

Equipment

- 1% starch solution
- 1% amylase solution
- Iodine solution
- 5 cm^3 syringe
- 10× boiling tubes
- 6× pipettes
- Spotting tile
- Water baths: 20, 30, 40, 50 and 60°C
- Stopwatch
- Permanent marker pen

Risk assessment

Hazard	Risk	Precaution	Emergency
Broken glass	Cuts	Keep glassware away from the edge of the desk	Dispose of broken glassware carefully; elevate cuts and apply pressure; do not remove glass from cuts; seek medical assistance
Boiling water	Scalding	Handle boiling water with care; use tongs to transfer boiling tubes; wear safety goggles	Run burn under cold water; seek medical assistance

Amylase solution	Irritation to eyes	Avoid contact with eyes; wear safety goggles	Flood eye(s) with tap water; seek medical assistance
	Irritation to skin	Wear gloves when handling amylase solution	Remove contaminated clothing; run the affected area under cold water; seek medical assistance
Iodine solution	Irritation to eyes	Avoid contact with eyes; wear safety goggles	Flood eye(s) with tap water; seek medical assistance

Method

1. Set up five water baths: 20, 30, 40, 50 and 60°C.
2. Using a pipette, place a **single** drop of iodine solution into each well of a spotting tile.
3. Set up five boiling tubes containing **2 cm^3 1% starch** solution and five boiling tubes containing **2 cm^3 1% amylose** solution.
4. Label each pair of boiling tubes with a temperature and place in the corresponding water baths for **10 minutes** to **equilibrate.**
5. Remove the boiling tube containing 1% starch from the 20°C water bath. Using a **5 cm^3 syringe**, add **2 cm^3** 1% starch into the 20°C 1% amylase solution. Start the stopwatch.
6. **Mix** using a pipette. After **30 seconds**, remove a sample of the reaction mixture using a pipette and place a drop of it onto the iodine in the first well. Record your observations.
7. Repeat this **every 30 seconds** until no further colour change is observed (the iodine remains yellow-brown). Note the time at which no colour change occurs.
8. Repeat steps 5 to 8 for the four other temperatures.
9. **Repeat** the method a further two times to obtain **three repeats** for each temperature.

Variables

Independent variable

The variable that is **changed**
i.e. temperature

Dependent variable

The variable being **measured** whose value depends on the independent variable i.e. the time taken for no further colour change.

Controlled variables

The variables that are kept **constant** during the experiment:

- Volume of iodine in each well of the spotting tile
  Pipette used to place one drop in each well
- Volume of 1% starch solution and 1% amylose solution
  5 cm^3 syringe used to measure 2 cm^3 of 1% starch solution and 1% amylose solution
- Concentration of starch solution and amylose solution
  1% starch solution and 1% amylose solution used throughout
- Time at which samples of the reaction mixture are removed
  Samples removed every 30 seconds (measured using a stopwatch)

Results

The initial rate of reaction is proportional to the reciprocal of the time taken for the reaction to be complete i.e. **1/t**. This allows values for the rate of reaction to be calculated for each temperature.

Temperature (°C)	Time taken for no further colour change (s)			Mean time (s)	Rate or 1/t (s^{-1})
	Repeat 1	Repeat 2	Repeat 3		
20					
30					
40					
50					
60					

A **graph** of rate of reaction against temperature can be plotted and the trend observed.

Conclusion

As **temperature increases**, the rate of reaction **initially** increases. This is because amylose and starch molecules have more **kinetic energy**. The **random movement** of molecules increases along with the probability of a **successful** collision. More **enzyme-substrate complexes** form so the rate of reaction increases.

However, **beyond the optimum** temperature, increasing vibrations **break bonds** in the enzyme's tertiary structure. The active site changes shape and the enzyme **denatures**. The rate of reaction decreases.